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OBSERVATIONS ON ERRANT FRUSTULES OF EUNOTIA MAJOR.

BY T. CHALKLEY PALMER.

The diatom *Eunotia major* Rab. occurs in some abundance in the uplands of Delaware Co., Pa., though appearing to flourish only in the basins of such springs as are well-shaded at all seasons. In these springs it is often present in profusion, forming masses of clean, brownish filaments attached to the sides of the pools, and by preference to submerged wood. The filaments grow to a length of ten centimeters, and vary in breadth from 0.036 mm. to 0.200 mm. in accordance with the length of their constituent cells. These filaments intertangle in such manner as to afford cover for numerous protozoa, and for such diatoms as *Surirella*, *Nitzschia*, *Pinnularia* and *Navicula*. *Eunotia lunaris* Grun. also frequently occurs in profusion in the same company.

In making a gathering of the filaments for study, it is well to begin by washing them free from all loosely adherent matters, such as sand, mica and clayey flocculence. They are then placed in a clean bottle which has been filled with water from the spring. A short exposure to diffused daylight will cause the diatoms to rise to the top, buoyed up by a multitude of oxygen bubbles entangled among them, and to free themselves from a further portion of mineral matter, which will at once subside, and may be rejected by decantation.

An examination of the gathering under the microscope will usually reveal, besides the filaments, a certain number of frustules, either isolated or in groups of two or three, moving about in a slow and erratic manner. In the course of two or three days, if all goes well, the proportion of moving cells will have increased; and from day to day, conditions permitting, the long filaments will progressively separate, until frequently the whole gathering ends by resolving itself entirely into isolated cells and short sections composed of two, four or even six cells, all in a state of lively locomotion.

Conditions favoring production of errant frustules.—The prime requisite for any extended study of the gathering is the preservation of the diatoms in a healthy condition. The best results have followed with *Eunotia major* when the clean filaments were kept in a semi-dark room, in a narrow-necked bottle filled with water from the native spring. Evaporation is slow and distilled water only is to be used in compensation. Any addition of town water is apt to be followed by a sudden and enormous increase of bacteria and a rapid decay of the diatoms. Bacteria do, in any case, gradually invade the culture and ultimately destroy it; but, with the above very simple precautions, it is quite possible to keep the gathering in good condition for a month—long enough to observe a variety of changes.

Free access of oxygen promotes the production of the motile frustules. Two approximately equal quantities of healthy filaments were selected, one of which was placed in a little vial with a narrow neck, the other in a flat glass dish ; and, other conditions, as temperature, light and amount of water, being identical, at the end of a week the filaments in the dish had entirely disappeared, and the glass was found to be coated with single cells and small motile segments of the original filaments, while the bulk of those in the vial remained unseparated.

A second experiment was as follows :—Two quantities of the unseparated filaments, as nearly equal as may be, were put into precisely similar bottles. The first was at once placed in a dark corner of a poorly lighted room, the second beside it, after having been exposed to bright daylight until a mass of oxygen bubbles had formed. In the course of twenty-four hours, during nine of which all light was absent, the exposed bottle contained quantities of active motile cells, while the other afforded only a very few. This experiment, twice repeated with the same result, taken in connection with that which precedes, would indicate that we have here to do with a process the reverse of the endothermic chlorophyl reaction :—

$$CO_2 + H_2O = CH_2O + {}_2O$$

and that the chemical side of the phenomenon is that which accompanies protoplasmic motile activity in general.

This conclusion is reënforced by the observed fact that filaments of *Eunotia* in process of separation, and the resultant motile cells, give a strong carbon dioxide color-reaction in thoroughly aerated water tinted with hæmatoxylin ; it is in accord with the important observation of O. Müller[1] as to the stimulating action of oxygen upon *Pinnularia;* and, finally, it is diametrically opposed to that view of the nature of diatom movements held by the proponents of the "osmotic hypothesis," especially as this is set forth quite recently by W. M. Kozlowski.[2]

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[1] Berichte der Deutsch. Botan. Gesellsch., Bd. XI, p. 571.

The stimulating action of light upon the motile activity of the errant frustules of *Eunotia* is not to be doubted, and has been observed a number of times; but, up to the present, notwithstanding that I have paid some attention to the matter, the phenomena observed are not such as are incapable of classification under the head of oxygen stimulation. No fact whatever has clearly indicated any special tendency to motion in the direction of greatest illumination, and after observing, under various conditions, the movements of thousands of these frustules, I have strong doubt as to the existence of any such tendency in *Eunotia*.

Characteristics of movements.—Students of diatom movements have, almost without exception, confined themselves to *Pinnularia* and other naviculoid forms wherein the phenomena are most striking. H. L. Smith,[3] and, more recently, O. Bütschli[4] and R. Lauterborn,[5] have been in practical agreement as to the phenomena, and to a certain extent they agree in the conclusion that motion is the result of an expulsion of a more or less fluid filament from the central nodule. O. Müller,[6] agreeing with the others as to the phenomena, looks rather, for their explanation, to currents of protoplasm which circulate along the raphe within the coleoderm, entering into and departing from the inner cell by way of the central and terminal nodules. Hauptfleisch[7] has studied forms of *Amphiprora* and *Brebissonia*, and by staining methods has apparently demonstrated in these the existence of a protoplasmic canal penetrating the raphe, through the delicate walls of which protrude exceedingly fine threads of protoplasm which act like similar organs in the rhizopods.

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[2] Botanical Gazette, Vol. XXIV, p. 39. Kozlowski presents in an ingenious manner considerations supposed to point to the conclusion that in *Pinnularia* motion is due to unequal photosyntax in the two ends of the frustule, causing stronger inward currents of assimilable liquid and gas in one or the other end, this difference depending upon the unequal illumination of the two ends.

[3] Proceedings Amer. Soc. Micros., 1886.

[4] Mittheilung über die Bewegung der Diatomeen, Heidelberg, 1892.

[5] Untersuchungen über Bau, Kerntheilung und Bewegung der Diatomeen, Leipsic, 1896.

[6] Berichte der Deutsch. Botan. Gesellsch.: Bd. VII, pp. 169-180: Bd. XI, p. 571.

[7] Mittheilungen des naturwiss. Vereins für Neu-vorpommern und Rügen, Jahrg., XXVII.

Little attention having been paid to the slower movements of forms outside the Raphidieæ, it is not unusual to meet with statements implying that definite and positive motion, requiring explanation, is not observable in the case of these forms. This is a curious error. Splendid exhibitions of motile activity have long been familiar in the case of various species of *Nitzschia,* and the exceedingly striking movements of *Bacillaria paradoxa* are known to observers the world over. Certain species of *Diatoma* and *Fragilaria* exhibit motile phenomena of no doubtful kind; and of *Eunotia major,* which may stand as a type of the Fragilarieæ, the following are the observed facts:—

When a drop of water containing errant frustules is observed under an enlargement of about 200 diameters, the first movement detected will probably be that illustrated in fig. 2, Pl. VI. Certain frustules rise slowly on their ends, rotating smoothly through 180°, and fall flat upon the slide. During this manoeuvre an end remains in contact with the glass and serves as a hinge whereon the frustule turns. Occasionally, but not often, a similar revolution is accomplished at right-angles to this—in which case the long valve, instead of the narrow end, functions as a hinge. This particular movement only occurs immediately after the deposition of the diatoms upon the slide, unless at this time the cells are not in a state of activity, in which case it may be somewhat delayed. A long series of observations, extending over three years, has shown both the conditions under which this odd movement occurs, and the manifold purpose of it. It is, first, significant that only those cells that have chanced to fall upon the slide with ventral or concave girdle uppermost, as in A, fig. 2, Plate VI perform this movement. Such as have been deposited with ventral side down, as in C, begin without delay to move endwise in the direction indicated in the figure. Careful focussing shows that in most cases the frustule is, during this forward movement, tilted a trifle, so that while the anterior end remains in contact with the glass, the posterior end is free from it. This position is undoubtedly a condition precedent to successful locomotion, and there is no doubt that the attaining of this position is the purpose of the rotation. That the anterior end is in contact with the slide will be proven conclusively as we proceed; and it is, therefore, to the end of the frustule, and more especially to the ventral side of the rounded end, that search must be directed for the mechanism of this diatom's movement.

The forward movement of *Eunotia* resembles very little the smooth gliding of *Pinnularia* and *Nitzschia.* If these seem to swim, the other would appear to crawl. One corner is advanced a trifle, then the other corner, then both move forward at once. Occasionally the frustule will sidle for a moment; but generally the progression consists of a succession of impulsive jerks. The movement is accompanied by indications of very considerable stress. Comparatively large bodies, such as short filaments of *Eunotia* or small particles of sand, are pushed resolutely aside. Larger bodies, especially such as have considerable thickness, arrest the frustule for a time; but after a short interval, it is apt to turn upon its edge, with ventral girdle in contact with the obstruction, to move past in this position, and once more to resume its normal relations with the slide. At the moment of the passage of the anterior, and sometimes the posterior end of the frustule, the obstruction may be seen to move backward, as if from an impulse resembling a kick. These curious features I have observed numberless times.

If by chance the moving cell pass, as in fig. 1, Pl. VI, with a corner over the circumference of a thin piece of mica, the latter is, in many cases, caused to revolve backward in the direction indicated in the figure. This also was seen so often that the existence of something resembling pseudopodia at the corners of the frustule came to be anticipated with confidence.

Many species of motile diatoms, among them *Pinnularia* and *Nitzschia,* exhibit a sort of pendulum movement. The frustule proceeds in a straight line a certain time, comes to rest, and returns upon its path without changing the direction of its longer axis. This swing to and fro is repeated any number of times, so that anterior and posterior ends continually change places. There is little of this in the movement of *Eunotia.* I have frequently followed a frustule for half an hour without seeing it return upon its path, and on one occasion I observed a particularly active individual for two and a half hours, in which period its devious way was into all portions of the hollowed slide, and in all that time it kept one and the same end foremost. Yet there seems to be no universal necessity for this habit, since frustules are frequently observed to exhibit propulsive efforts upon solid bodies from both ends, and occasionally they do reverse their direction.

Movements in the hanging drop.—In order to study somewhat further the relations between moving *Eunotia* and the glass of the support, drops of water containing many frustules were suspended on cover-glasses and observed. The diatoms were found to separate into two portions. The first remained attached to the glass; such of them as had their dorsal or convex girdles next the glass performed the revolution before described; and these, with the others in contact with the cover, proceeded in characteristic ways, with the curious and significant difference, that as viewed from above the ventral side was now uppermost. The second portion fell to the surface of the suspended drop, where they moved as if upon a solid substratum. Midway of the drop there were none. Undoubtedly the surface of the drop possessed sufficient tension for the purposes of a crawling diatom. I have seen, in an aquarium, small snails crawling freely along the plane of contact between water and air, with broad, flat foot uppermost, applied to the surface of the liquid as if to a piece of glass.

Phenomena in carbon emulsion.—All preceding observations seeming to point to the existence of invisible organs functioning as pseudopodia, the endeavor was made to outline these by the well-known method of putting the diatoms in an emulsion of colored particles finely divided. To this end, since available India ink was found to be toxic owing to its content of camphor, a portion of carbon-black water-color was rubbed up in a drop of water until a very dark color was obtained. The emulsion showed, under medium powers, a multitude of particles exhibiting the Brownian movement. When placed in this, the errant *Eunotia* cells revealed quite clearly the existence at the corners of rounded masses of coleoderm. These were especially clear at the anterior corners. The particles of carbon approached the frustules quite closely everywhere except about the corners, and a little U-shaped line, composed of somewhat compacted particles, preceded the moving diatom. There was nowhere observable any streaming away of the particles, such as that seen by H. L. Smith and others in *Pinnularia.* Owing to the tenacity of the emulsion, and partly perhaps to the relatively raised position of the posterior end of the frustule, the clear spaces are less evident at the posterior corners.

Results of staining.—Numerous attempts were made with a variety of stains to render the coleoderm visible, the presence of which seemed to be so strongly indicated. All efforts to stain the still living coleoderm failed entirely. There was little better success in fixing with osmic acid and staining with methylene blue, methyl blue, fuchsin, etc., for the osmic acid seemed to produce, as it were, a certain shrinking of the very delicate organs. A measure of success followed the use of gentian violet followed by tannic acid, both in minute quantity. By this means the coleoderm was outlined; and especially in the case of frustules that had passed the period of their activity, and were nearly or quite dead, as shown by their contracted nucleus and bacteria-infested outline, the surface staining of the gelatinous external layer was clear. An extreme case of this is roughly shown in fig. 7, Pl. VI. Here the contents of the inner cell were apparently normal, with the exception of the nucleus, but the coleoderm was quite flaccid, loosely adherent to the frustule, and abnormally enlarged, as well as fringed with colonies of bacteria.

The stain which most quickly and surely shows the healthy coleoderm, at the same time instantly killing the diatom, is made as follows:—0·5 gram of Bismarck brown and 1.0 gram tannic acid are dissolved separately and added to a liter of distilled water. The solution remains perfectly clear, and is of a reddish-brown color. Two or three drops of this are added to a drop of water, under the cover glass, containing errant frustules of *Eunotia*. Almost before a change of tint is visible in the thin layer of water under the microscope, motion ceases, and, at the same time, at each corner of the frustules appears a little rounded mass of substance, gelatinous in appearance, and dotted with coppery or bronzy specks of most minute size. The shape of these masses, and their relation to the frustule, are indicated in figures 4 and 5, Pl. VI. The results of somewhat heavier staining with the same mixture are shown photographically by Mr. F. J. Keeley in figures 4, 5, and 6, Pl. VII.

Pursuant to the further study of these coleoderm masses, a large number of errant frustules were stained as follows: About two drops of water, containing the diatoms, were put on a cover glass and allowed some minutes to bring themselves into normal relations with the glass. Three drops of the described stain solution were added and staining was allowed to proceed half an hour. The excess of color was then removed by careful washing, dipping the cover repeatedly into a cup of water with as little friction as possible. Grübler's aqueous eosin, diluted with an equal quantity of water, was now added and allowed to act half an hour to an hour. Finally, the glass was washed and mounted in very weak formalin. The diatoms, under these circumstances, remain attached to the cover glass, as a rule with ventral side uppermost, and with coleoderm processes in the position best suited for study.

Prepared in this manner, the isolated cells and short segments of filaments present the appearance indicated by figures 3 to 5, Pl. VI. The gradual development of the foot-like organs from an intercellular plasm is shown in figure 3; and in figure 6 is shown the deterioration of these organs, which takes the form of an overproduction and liquefaction of gelatinous substance, and ends by gradually bringing to rest all the moving cells of a gathering, and entangling them in masses and threads of jelly. During this degeneration many of the cells die, but some survive with every appearance of health, though necessarily devoid of motion.

In figures 8 and 9 is shown the appearance, under 1,000 diameters, of the stained processes from the valve view and from the ventral girdle view. The outer and larger process, mainly gelatinous, shows a general mass slightly tinted only, outlined by more heavily stained grains within the substance. These grains take the eosin as do the protoplasmic granules of the inner cell. The smaller process, which seems to be somewhat shrunken by the staining with eosin (compare figures 4, 5 and 6, Pl. VII) are of a deep and nearly uniform red, and are in close apparent contact with aggregations of protoplasm within the frustule.

The small and apparently constant bead-like bodies around the circumference of the curving gelatinous masses have no perceptible thread-like connection with the more richly protoplasmic processes at the corners, and their significance is somewhat doubtful. They probably have some relation to a current of protoplasm which would seem to issue from the cell and return to it.

Structure oj the frustule.—The existence of a "pseudo-nodule" on the ventral side of the valve, near the end, has long been known; but that there is a raphe at the end of the frustule has not, I believe, been stated. The photographs in Plate VII, which I owe to the kindness of Mr. F. J. Keeley, show clearly both the existence of this raphe and the presence, especially on the ventral girdle, of several channels in the silica which seem adapted to lead currents to and from this raphe and the pseudo nodule.

The exact shape of the raphe itself, and its extent, remain to be determined; and indeed the problem presents difficulties, for the position of the raphe on the end of the frustule would necessitate sections for its study.

When a very dilute aqueous solution of gentian violet is dropped upon living and moving *Eunotia major*, and afterward washed off, certain deeply stained lines are seen in immediate contact with the silica of the ventral surface, and running down from the pseudonodule and the end nodule. These dark lines follow the course of the channels in the silica shown by the photographs, and would be explained by the existence of richly protoplasmic fluid. In a single case one of these lines was seen to issue, to all appearance, through the raphe and enter the coleoderm.

The above results of observation and experiment would seem to me to be conclusive proof that *Eunotia major*, and by inference other nearly related species and genera of the *Fragilarieæ*, move by the action of organs that may be càlled coleopodia. This conclusion, however, is far from touching the question of the means of locomotion in *Pinnularia* and its allies, and I have endeavored to indicate some of the differences of the phenomena to be explained in the two cases. It may be added in this connection that while a large *Pinnularia* in rapid motion not infrequently gives evidence of brisk internal currents such as O. Müller has described, such currents have not been observed by me in moving *Eunotia*. Under rather high powers one only sees, near the corners of the frustule, in the vicinity of the raphe, a certain internal commotion among the very small protoplasmic granules, a spasmodic movement back and forth, a weaving about. This movement differs alike from the streaming of cyclosis and the Brownian trembling, and it is traceable with difficulty, if at all, far from the corners. Nevertheless, the channelling of the frustule is of a character to indicate the existence of currents, and further observation may yet reveal them.

EXPLANATION OF PLATES.

Plate VI. diagrammatic.

Fig. 1.—Representation of *Eunotia major* moving across a piece of mica and revolving it in the opposite direction.

Fig. 2.—A frustule, valve view, in A resting upon its dorsal girdle, B revolving, and C reaching its normal motile position and moving in the direction of the arrow.

Fig. 3.—A short filament showing stages in the development of the coleopodia, and a free motile cell with development complete. Stained with Bismarck brown and eosin.

Fig. 4.—Valve view of motile cell, stained with Bismarck brown and eosin.

Fig. 5.—Stained cells, including a group of two.

Fig. 6.—Degeneration of the coleopodia, from overproduction of gelatinous substance, resulting in adherence. Stained as before.

Fig. 7.—Degeneration of the whole coleoderm, which is infested with bactaria in colonies. Gentian violet.

Figs. 8 and 9.—Coleopodia under higher magnification, showing differential staining by Bismarck brown and eosin.

PLATE VII.

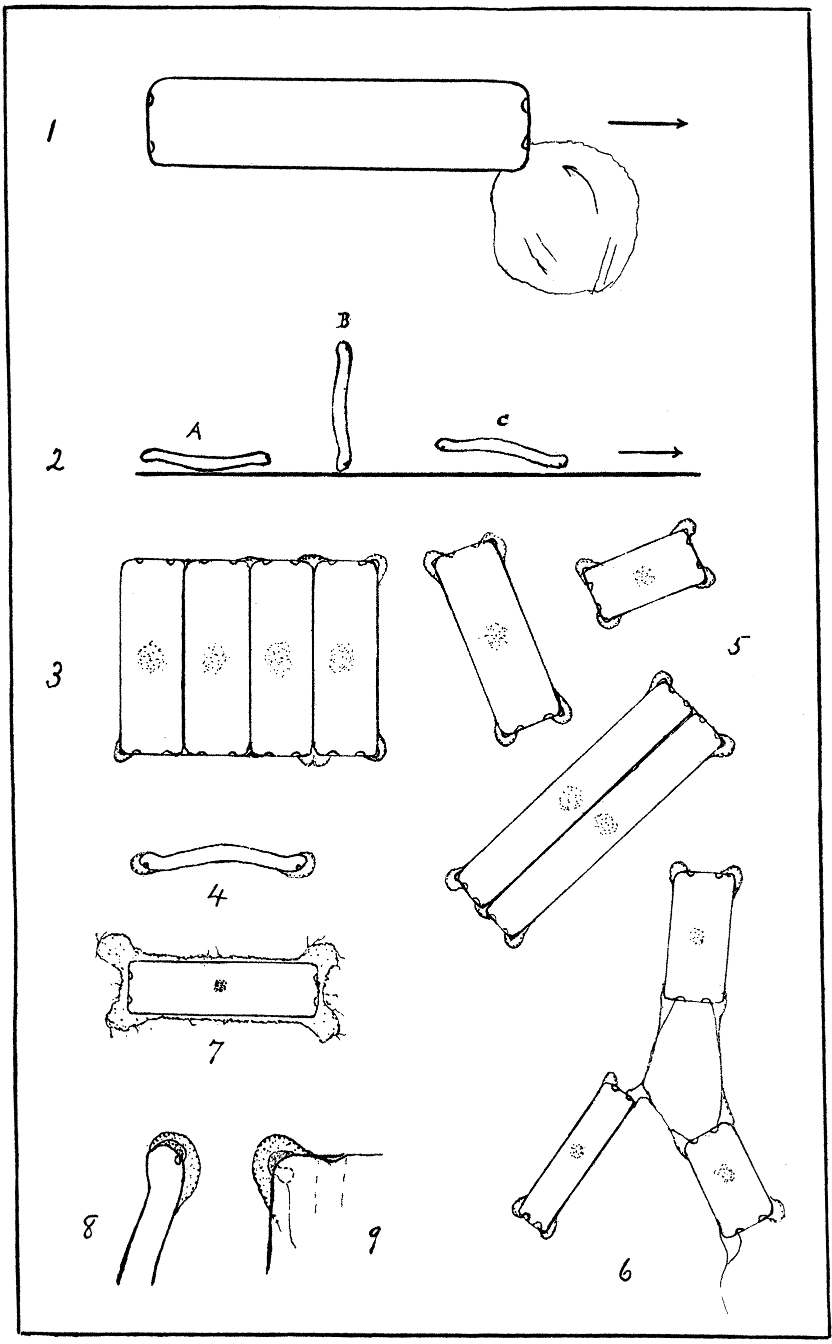
Fig. 1.—Ventral girdle view of cleaned frustule of *Eunotia major* × 925.

Fig. 2.—Optical section of the same frustule, showing the end raphe and channels leading to raphe and pseudo-nodule, × 925.

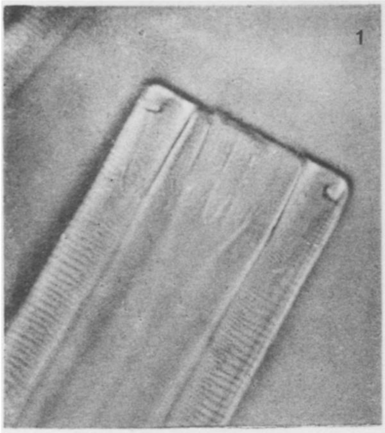
Fig. 3.—Dorsal girdle of the same frustule, × 925.

Figs. 4 and 5.—Motile frustules of *Eunotia major*, stained with Bismarck brown and tannic acid, × 315.

Fig. 6.—An enlargement of 4 (× 435).



PALMER. MOVEMENTS OF EUNOTIA.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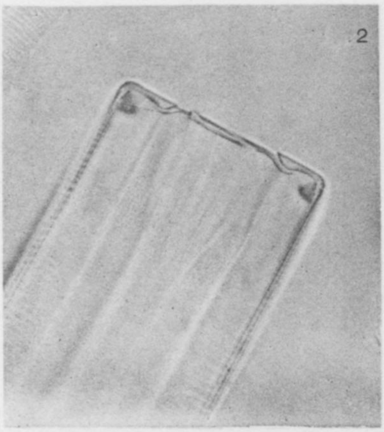
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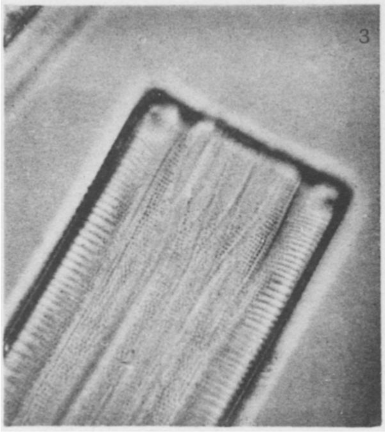
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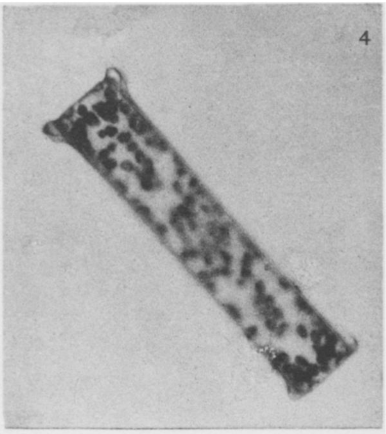
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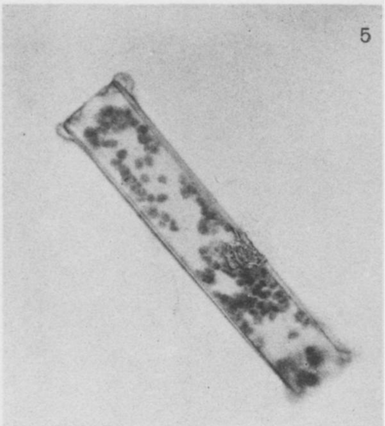
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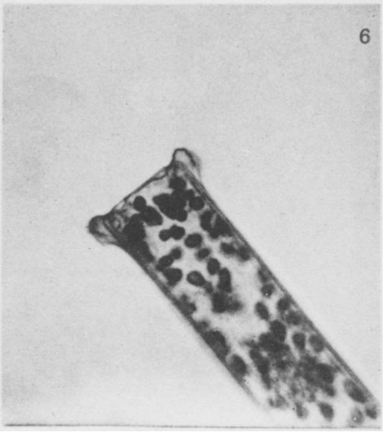
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